



Interactive effects of elevated temperature and pCO₂ on early-life-history stages of the giant kelp *Macrocystis pyrifera*



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ARTICLE INFO

Article history:

Received 26 December 2013

Received in revised form 23 March 2014

Accepted 24 March 2014

Available online xxx

Keywords:

Macrocystis pyrifera

Climate change

Ocean acidification

Global warming

Synergistic effects

Non-calcifying algae

ABSTRACT

Rising atmospheric CO₂ is expected to increase global temperatures and partial pressure of CO₂ in surface waters, causing ocean warming and acidification. These changes may have important consequences for the physiological performance of early life-history stages of marine organisms. In this study we investigated the potential for interactive effects of ecologically relevant levels of temperature and pCO₂ on germination, dormancy and mortality of zoospores of the giant kelp *Macrocystis pyrifera*, a foundation species of temperate reef ecosystems. Newly settled kelp spores were cultured in the laboratory for seven days in a factorial design with temperature (13 °C and 18 °C) and pCO₂ (~370 and ~1800 μatm) as experimental factors. The two levels of temperature and the low-pCO₂ treatment in our design were consistent with present-day environmental conditions in the kelp forest as measured by autonomous temperature and pH sensors, while the high-pCO₂ treatment reflects an extreme, future acidification scenario. Our results revealed that the combined effects of increased temperature and pCO₂ can significantly decrease germination rates and increase the mortality of kelp spores. Interactive effects of temperature and pCO₂ were detected on spore mortality and dormancy. Spore mortality only differed between pCO₂ treatments at high temperature. In contrast, spore dormancy was higher in the treatment with low temperature and high pCO₂, which is similar to the environmental conditions experienced during upwelling events in southern California. Our results highlight the importance of considering multiple stressors to understand how the early-stages of foundation species such as *M. pyrifera* will be affected by global change.

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1. Introduction

Broad-scale environmental changes, such as ocean warming and ocean acidification (OA), have been subjects of intense research in an ecological and evolutionary context due to their expected deleterious impacts on marine ecosystems (Etterson and Shaw, 2001; Davis et al., 2005; Bell and Collins, 2008; Halpern et al., 2008; Doney et al., 2009; Dillon et al., 2010; Gruber et al., 2012; Kelly and Hofmann, 2013; Kroeker et al., 2013; Poloczanska et al., 2013). Since the Industrial Revolution, atmospheric CO₂ levels have increased from 280 to ~392 ppm (Raven et al., 2005), and it is predicted that atmospheric pCO₂ will continue to increase at a rate almost 100 times higher than anytime in the last 650 thousand years (Siegenthaler et al., 2005). Approximately 1/3 of the excess of CO₂ in the atmosphere will be absorbed by surface ocean waters, leading to an estimated drop in pH of 0.3–0.4 units

(pCO₂ ~ 1000 μatm) globally by the year 2100 (IPCC, 2013) and up to 0.8 units (pCO₂ ~ 2000 μatm) by the year 2300 (Caldeira and Wickett, 2003, 2005; IPCC, 2013). At a local level, the effects of the OA are predicted to be more severe, especially in coastal regions (although see Duarte et al., 2013) with upwelling of CO₂-rich water and pCO₂ (i.e., CO₂ partial pressure) values above 4000 μatm (Hales et al., 2005; Hauri et al., 2009; Thomsen et al., 2010; Gruber, 2011). In addition to OA, rising atmospheric greenhouse gas concentrations have increased global average temperatures by ~0.2 °C per decade over the past 30 years (Hansen et al., 2006). At this rate, the estimate for increase in mean sea-surface temperatures by 2100 is predicted to lie between 1.4 and 5.8 °C (IPCC, 2013).

A large body of empirical evidence has shown the effects of ocean warming and acidification on the physiology (Pörtner, 2008; Hofmann and Todgham, 2010; Whiteley, 2011; Padilla-Gamiño et al., 2013), calcification (Hofmann et al., 2010; Walther et al., 2011), development and growth (Kurihara, 2008; Byrne et al., 2013; Yu et al., 2013), and reproduction (Havenhand and Buttler, 2008; Byrne, 2011; Sewell et al., 2014) of marine organisms, most of them marine calcifiers such as sea urchins, coralline algae, mussels and corals (Hofmann et al., 2010). However, little is known about the effects of increased

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temperature and pCO₂ in non-calcifying marine species such as the giant kelp *Macrocystis pyrifera*. This seaweed is an important ecosystem engineer and is the most widely distributed kelp species in temperate waters of the Northern and Southern hemispheres (Graham et al., 2007). *M. pyrifera* has a heteromorphic life history with an alternation of macroscopic and microscopic generations (Fig. 1) (Graham et al., 2007; Roleda et al., 2012). The reproductive life cycle is initiated in specialized macroscopic blades called sporophylls (Fig. 1). Meiosis produces biflagellate zoospores (~6–8 μm length) that are released into the water column where they disperse via currents until they reach suitable substrata where they settle and germinate (Graham et al., 2007). Successful spore germination produces microscopic haploid male and female gametophytes (~80–400 μm) which produce sperm and eggs, respectively (Fig. 1). During these cryptic microstages, *M. pyrifera* has been shown to be susceptible to environmental stressors such as those induced by climate change (Coelho et al., 2000; Roleda et al., 2012; Buschmann et al., 2013), which can interfere in the completion of its life cycle and affect recruitment in natural populations (Graham et al., 2007). For example, experimental evidence suggests that high temperatures and low nutrient concentrations associated with El Niño Southern Oscillation (ENSO) inhibit development of haploid microstages of *M. pyrifera* (Ladah and Zertuche-González, 2007). Similarly, in a recent study, Roleda et al. (2012) found that low pH values (in absence of additional dissolved inorganic carbon), associated with predicted scenarios of OA, produce a significant reduction on zoospore germination in this marine brown alga.

Here, we investigated the potential for interactive effects of ecologically relevant levels of temperature and pCO₂ on germination, dormancy and mortality of early-life-history stages of the giant kelp *M. pyrifera* from southern California. We also investigated the effect of a higher pCO₂ level that would reflect future OA in the kelp forest. These kelp populations produce spores throughout the year (Reed et al., 1996) and are exposed to highly pronounced seasonal changes in environmental characteristics (Lynn and Simpson, 1987; Yu et al., 2011) with temperature regimes that reflect average warming trends in the global ocean (Field et al., 2006) and considerable declines in pH as a result of upwelling of CO₂-rich waters (Hauri et al., 2009; Gruber et al., 2012). Because predicted future scenarios indicate a threat to marine life through the direct or synergistic interaction of OA and ocean warming

(Byrne et al., 2013), we hypothesized that early-life-history stages of *M. pyrifera* would be negatively affected by the synergistic effects of decreased ocean pH and elevated temperature, evidenced by suppressed zoospore germination and increased mortality.

2. Material and methods

2.1. Algal material

Reproductive blades (sporophylls) were selected from seven adult sporophytes of *M. pyrifera* in kelp beds near Santa Barbara, California (34°25'N, 119°57'W) in April 2013. Because genetic differentiation is positively correlated with geographical distance and occurs at spatial and temporal scales in *M. pyrifera* (Alberto et al., 2010), we selected individuals from seven different sites within the Santa Barbara channel in order to obtain different genotypes from the population. Sporophylls were collected by divers using SCUBA at depths of about 10 m, placed in plastic containers, and immediately returned to the aquarium facilities at UC Santa Barbara. Spores were obtained following methods described in Reed et al. (1992). In brief, sporophylls were cleaned of epibionts, rinsed with filtered seawater, blotted dry, and kept in darkness on moist paper towels at 5 °C for one hour. To induce spore release, sporophylls from each sporophyte were separately immersed in 0.2 μm-filtered, UV-sterilized seawater (FSW) at room temperature under low light. After 10 min, spore suspensions were filtered through a 20 μm mesh screen to remove debris and placed in 1 L beakers with a total volume of 500 mL of FSW. Concentrations of suspended spores generated from each sporophyte were quantified using a hemocytometer, and diluted with FSW (T = 14.5 °C, pH = 7.8, pCO₂ = 421.5 μatm and salinity = 33.2 ppt) to 5 × 10⁴ cells mL⁻¹ into 50 mL Petri dishes (n = 2 dishes per adult sporophyte). Spores were allowed to settle for 1.5 h, after which the Petri dishes were gently washed and filled with the corresponding treatment seawater (Table 1) mixed with Provasoli medium (PESI) (Provasoli, 1969) in a ratio of 10 mL of PESI:1000 mL of seawater.

2.2. Experimental cultures

To determine the effects of temperature and pCO₂ on early-life-history stages of the giant kelp *M. pyrifera*, Petri dishes containing

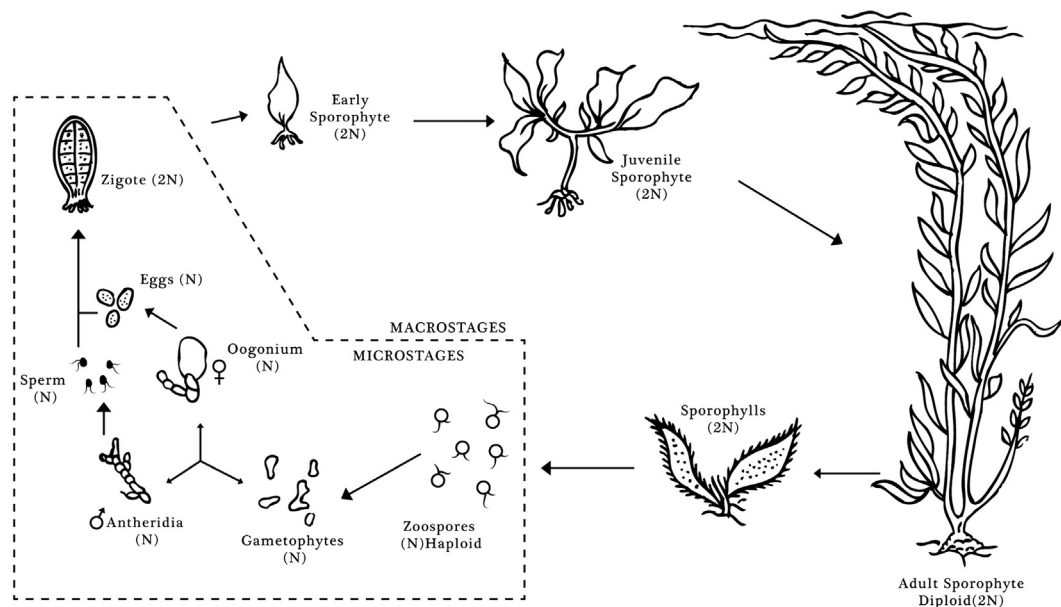


Fig. 1. *Macrocystis pyrifera* life cycle depicting various life-history stages with important implications in local population dynamics. 2N is the diploid number of chromosomes, and N is the haploid number. Early developmental microstages are shown in the dashed box. Modified from (North, 1987).

Table 1
Summary of seawater chemistry for the experimental treatments of kelp spores.

Treatment	Low pH	High pH
Salinity (ppm)	33.2 ± 0.3	33.2 ± 0.3
pH	7.46 ± 0.03	7.97 ± 0.04
pCO ₂ (µatm)	1790.5 ± 78.4	370.8 ± 34.6
TA (µmol kg ⁻¹)	2223.5 ± 4	2219.4 ± 7
Ω _{ara}	0.79 ± 0.04	2.14 ± 0.11
Ω _{cal}	1.29 ± 0.05	3.37 ± 0.28

settled spores from the seven sporophytes were incubated in a short-term experiment (7-days) with a factorial design of temperature (13 °C and 18 °C) and pCO₂ (~370 and ~1800 µatm; Table 1). All experimental treatments were performed in duplicate. Petri dishes were filled to minimize headspace, tightly sealed using Parafilm™, and treatment seawater was replaced every 3 days. A preliminary experiment using treatment seawater with and without algal spores showed no change of pH in the culture media after 3 days. Petri dishes were kept inside a thermal incubator (1585 VWR Shaking Incubator) and provided irradiance of 43.3 ± 6.6 µmol photon m⁻² s⁻¹ (SE) under a 14 h light: 10 h dark photoperiod using LED lights (MarineLand Reef).

Germinated, dead and ungerminated (as a proxy of resting or dormant) spores were counted at the seventh day of the experiment using the methods of [Roleda et al. \(2012\)](#). Five randomly chosen visual fields of each Petri dish were observed under 20× magnification (>300 spores per replicate) using an Olympus IMT2 inverted microscope (Olympus Corp., Tokyo, Japan). Kelp spores were considered germinated when a distinct germ tube could be seen ([Roleda et al., 2012](#)) and dead when they exhibited no pigment and hollow opaque cells ([Roleda et al., 2004](#)). Although we were not able to recognize metabolically active or inactive spores, we defined dormant spores as those cells that exhibited pigmentation and undisrupted shape at the end of the experiment, but that lacked development of germination tubes, which are produced by active cellular metabolism ([Amsler and Neushul, 1991](#); [Agrawal, 2009](#)).

2.2.1. Seawater treatment

Experimental treatments of high and low seawater pH were created using a flow-through CO₂ mixing system ([Fangue et al., 2010](#)). Low-pCO₂ treatments (Table 1) were based upon environmental data recorded using autonomous pH sensors deployed at the site of kelp collection in the Santa Barbara Channel (34°46'N, -120°12'W). The high pCO₂ treatment reflects extreme records reported for natural environmental conditions during upwelling events ([Hofmann et al., 2011](#)); in this case, ~1800 µatm of pCO₂ (pH ~7.5) was the high exposure level and ~370 µatm (pH ~8.0) was used to represent the lower exposure level. Temperature levels were chosen to represent present-day seasonal variation in kelp forests off Santa Barbara, CA, USA ([Fram et al., 2008](#)). Temperature, salinity and pH were measured daily for each pCO₂ system according to best-practice procedures ([Dickson et al., 2007](#); [Fangue et al., 2010](#)). Temperature was measured using a wire-thermocouple (Themolyne PM 207000/Series 1218), and salinity was measured using a conductivity meter (YSI 3100). pH was determined following the standard operating procedure (SOP) 6b ([Dickson et al., 2007](#)) using a spectrophotometer (Bio Spec-1601, Shimadzu) and dye m-cresol purple (Sigma-Aldrich) as the indicator. Total alkalinity (TA) was measured every three days in the reservoir buckets, following the SOP 3b ([Dickson et al., 2007](#)). Both pH and alkalinity were assessed for accuracy using certified reference materials (CRMs) from Dickson (Scripps Institution of Oceanography), Batch 8 (pH = 8.0923 + 0.0004) and Batch 103 (TA = 2232.94 + 0.79 mmol kg⁻¹) for pH and alkalinity, respectively. Parameters of pCO₂, Ω_{ara} and Ω_{cal} were estimated using CO2calc ([Robbins et al., 2010](#)) with the carbonic acid dissociation constants of [Mehrbaach et al. \(1973\)](#).

2.3. SeaFET deployment

An autonomous pH sensor ([Martz et al., 2010](#)), called a SeaFET, and a CTD profiler (Seabird Electronics) were deployed at the Arroyo Quemado site maintained by the Santa Barbara Coastal Long-Term Ecological Research program (34°46'N, -120°12'W). This site is characterized by a kelp forest surrounded by sand flats at 10 m depth. The sensors, mounted on a mooring cage at 10 m, recorded pH, temperature, and salinity at 20-minute intervals. Two consecutive deployments generated data between July 30, 2012 and January 17, 2013. Raw sensor data were calibrated with seawater field samples collected by SCUBA divers and a Niskin bottle. Calibration samples were preserved with mercuric chloride prior to analysis. pH, salinity, and total alkalinity were measured for these samples as described above. The time series of raw sensor data (voltage) was converted into seawater pH (total scale) using SeaFET temperature data, calibration sample pH, and CTD salinity. Then, pCO₂, Ω_{ara} and Ω_{cal} were calculated using CO2calc ([Robbins et al., 2010](#)) using carbonic acid dissociation constants of [Mehrbaach et al. \(1973\)](#).

2.4. Statistical analysis

Percentages of germinated, ungerminated, and dead spores were averaged for the two Petri dishes of each sporophyte, and the means for each sporophyte were treated as replicates for all analyses (n = 7). Prior to analyses, we tested for normality and homoscedasticity for all variables using the Lilliefors and Levene tests, respectively. Percentages of germinated, dormant, and dead spores were log₁₀-transformed before the analysis. In order to evaluate the interactive effect of temperature and pCO₂ on all variables measured, two-way ANOVAs were used with experimental temperature and pCO₂ as fixed factors. When the analysis did not show significant interactions, a one-way ANOVA was carried out for each factor separately. In all cases, when differences in the means were significant at the P < 0.05 level, they were also tested with a posteriori Tukey's test (HSD). All analyses were run in the Statistica v7.0 software (StatSoft).

3. Results

3.1. SeaFET data

Carbonate chemistry at Arroyo Quemado varied around a mean pH of 8.041 ± 0.042 and a mean pCO₂ of 400 ± 48 µatm (Supplementary Table 1). During the deployments, pCO₂ reached a maximum of 777 µatm (pH = 7.779). The low-pCO₂ treatment used in this experiment (371 µatm) is similar to present day conditions (average pCO₂ = 476 µatm) and within the range of documented exposures at this location (301–777 µatm pCO₂). Temperature oscillated between 21.74 and 11.54 °C, with a mean of 16.53 °C ± 1.94 °C. The high temperature treatment reflected temperatures during the period from August to October ([Fig. 2](#)). The low temperature treatment for this experiment (13 °C) was lower than the average environmental temperature recorded for this time period (16.53 °C) but matched the environmental temperatures experienced in Winter 2013 ([Fig. 2](#), Supplementary Table 1). During January, the sensors showed a sharp decrease in temperature and pH ([Fig. 2](#)), which may indicate upwelling events of cold acidic waters. Salinity remained fairly constant (~33.5 psu) during the deployments, varying by only 0.5 psu ([Fig. 2](#), Supplementary Table 1).

3.2. Seawater parameters and kelp spore culture

Temperature and carbonate seawater chemistry remained stable during the experimental period with pCO₂ levels close to the selected values (see [Table 1](#)). Initial development of germination tubes was observed in some cases after 20 h. After seven days under experimental conditions, there were clear differences in morphology and coloration between germinated, dormant (i.e., ungerminated) and dead spores.

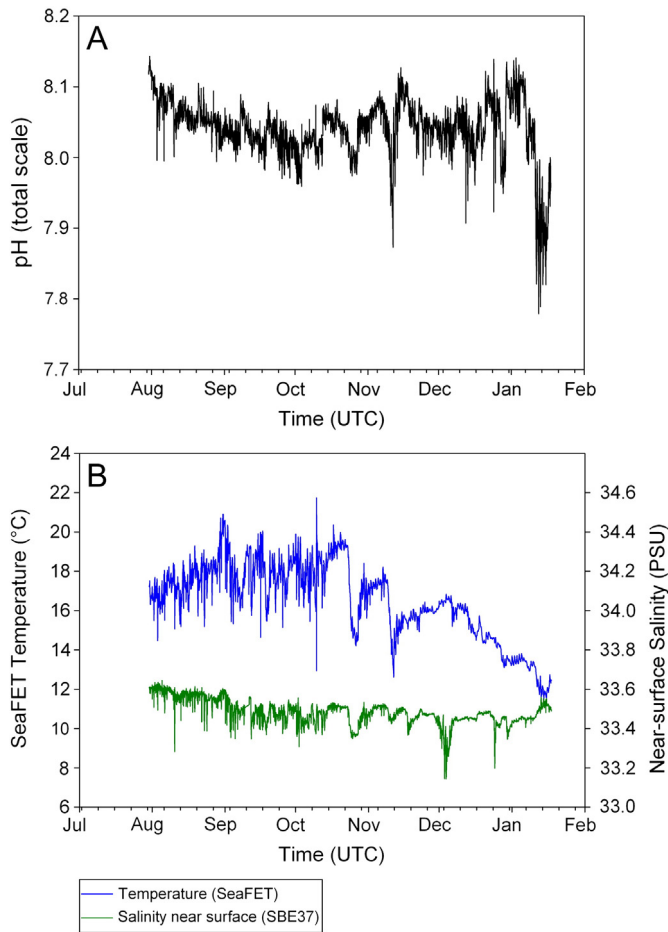


Fig. 2. Time series of pH (A), temperature and salinity (B) from field deployment (July 30, 2012 and January 17, 2013) of SeaFET and Seabird CT sensors. Temperature is shown in blue on the left y-axis whereas salinity in green on the right y-axis.

3.3. Effect of temperature and $p\text{CO}_2$ on kelp spores

Both, temperature and $p\text{CO}_2$ had significant effects on germination rates ($F_{1,26} = 150$, $P < 0.001$, $F_{1,26} = 292$, $P < 0.001$, temperature and $p\text{CO}_2$ respectively). Overall, germination rates were ~30% higher in the low temperature treatment of 13 °C compared to the high temperature treatment of 18 °C (Fig. 3A). At both temperatures, spores in the high $p\text{CO}_2$ treatment had lower germination rates than spores in the low $p\text{CO}_2$ treatments (~54% and ~66% respectively). The highest germination rates (~78%) were observed in the low temperature/low $p\text{CO}_2$ treatment and the lowest germination rates (~35%) were observed in

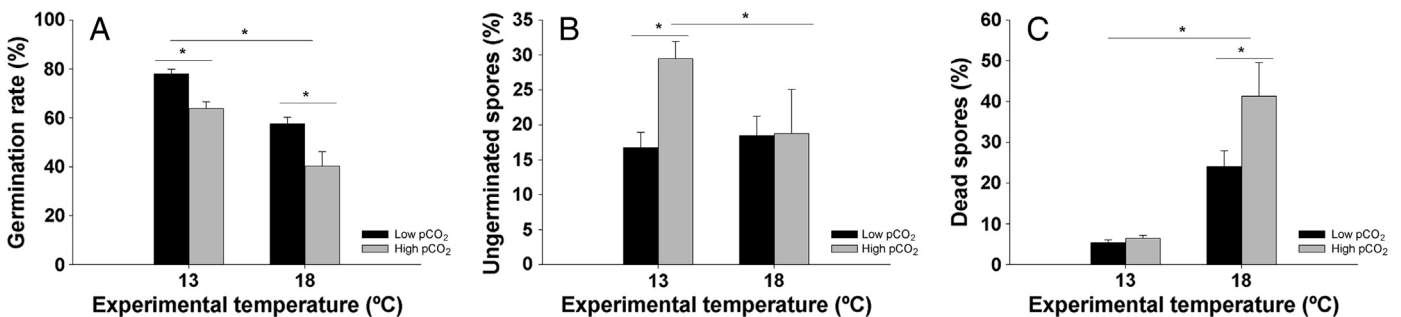


Fig. 3. Germination rate (A), dormancy (B) and mortality (C) of *Macrocytis pyrifera* spores under thermal (low and high temperatures, 13 °C and 18 °C respectively) and pH/ $p\text{CO}_2$ (~7.5 and ~8.0/~1800 μatm and ~370 μatm respectively) treatments. Values are adjusted means \pm S.E.M. Asterisks indicate statistical significance.

the high temperature/high $p\text{CO}_2$ treatment. There was no significant interaction between temperature and $p\text{CO}_2$ for germination rates ($F_{1,24} = 1.4$, $P > 0.24$).

The effects of $p\text{CO}_2$ on the percentage of ungerminated (dormant) spores varied with temperature (temperature \times $p\text{CO}_2$ $F_{1,24} = 16.12$, $P < 0.001$). Specifically, at 13 °C, the percentage of ungerminated spores at high $p\text{CO}_2$ was twice that at low $p\text{CO}_2$, whereas $p\text{CO}_2$ had no effect on the percent germinated at 18 °C (Fig. 3B and Fig. 4).

Interactive effects between temperature and $p\text{CO}_2$ were also detected in spore mortality ($F_{1,24} = 21.9$, $P < 0.001$). Mortality was <10% at 13 °C regardless of the concentration of $p\text{CO}_2$ (Fig. 3C and Fig. 4). In contrast, spore mortality was >20% at 18 °C and twice as high in the high $p\text{CO}_2$ treatment compared to the low $p\text{CO}_2$ treatment (Fig. 4).

4. Discussion

Early-life-history stages of marine organisms often are considered to be population bottlenecks due to their high susceptibility to environmental stressors (Underwood and Fairweather, 1989). The main goal of this study was to test whether this was true for a macroalga by exploring how multiple global change stressors would affect early life stages of *M. pyrifera*. Our results show that the combined effects of increased temperature and $p\text{CO}_2$ can significantly decrease developmental rates and survivorship of kelp spores.

4.1. Environmental pH data

In order to better frame our experiment and to use environmentally-relevant $p\text{CO}_2$ values, we deployed pH, temperature, and salinity sensors near kelp forests in the Santa Barbara Channel. The pH and temperature time series generated during July 2012–January 2013 confirm that the experimental conditions used in this study represent present-day abiotic exposures as well as a future scenario of acidification and warming of the nearshore region of the California Current Ecosystem (Feely et al., 2008; Gruber, 2011). The variability in pH differs between sites throughout the Santa Barbara Channel (Hofmann et al., 2011, Santa Barbara Channel LTER data, unpubl.) and likely throughout the biogeographic range of *M. pyrifera*. Overall, large fluctuations in temperature and pH observed during January may be the result of upwelling events of cold acidic waters that are natural phenomena in the North Pacific coastal area (Feely et al., 2008). Other studies also have documented intervals of active upwelling during mid-January in the Santa Barbara Channel (Bringué et al., 2013), which may be related to the beginning of the transition to higher wind stress that occurs during February through March (Brzezinski et al., 2013). However, these fluctuations were smaller than those described during the spring upwelling (Frieder et al., 2012), which is the period of intense upwelling in the Santa Barbara coastal region (Feely et al., 2008; Yu et al., 2011; Brzezinski et al., 2013).

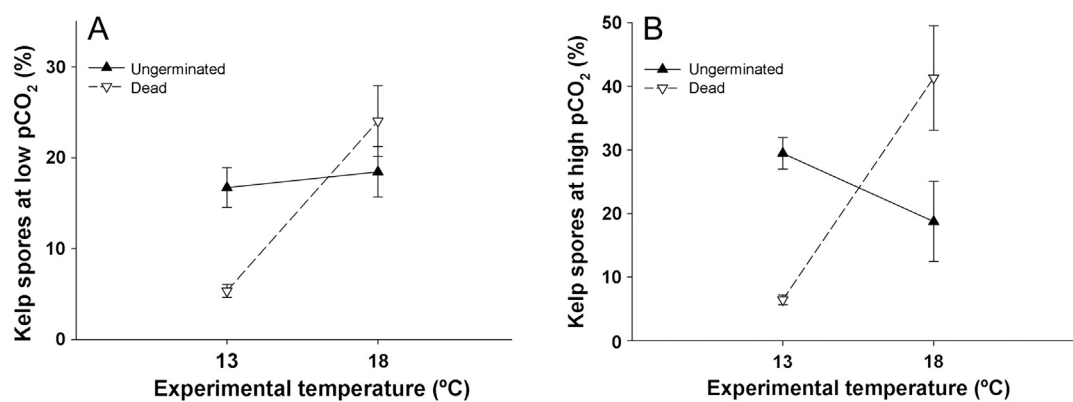


Fig. 4. Reaction norms for dormancy (ungerminated spores) and mortality (dead spores) in early-life-history stages of the giant kelp *Macrocystis pyrifera*. A) low and B) high pCO₂ treatments (pH/pCO₂: ~7.5 and ~8.0/~1800 μ atm and ~370 μ atm respectively). Values are adjusted means \pm S.E.M.

4.2. Germination

Development of germination tubes was affected by temperature and pCO₂. The lowest germination rates (~35%) were found in the high temperature treatment (18 °C). Drastic effects of temperature on germination, development and growth have been also documented in other kelp species. For example in *Alaria esculenta*, the germination capacity of zoospores decreased at temperatures higher than >16 °C, and microscopic stages were more sensitive than the adult macroscopic stages to temperature (Fredersdorf et al., 2009). Similarly, Müller et al. (2008) found that zoospores of some Arctic and temperate kelp populations of *Saccharina latissima*, *Laminaria digitata* and *Laminaria hyperborea* exhibited inhibition or decreased germination at 18 °C than at lower temperature (Müller et al., 2008). Moreover, in *L. saccharina*, temperatures between 17 °C and 20 °C were unfavorable for development of young female gametophytes and resulted in greater numbers of male gametophytes as a population response to unfavorable environmental conditions (Lee and Brinkhuis, 1988). In our study, the decrease of zoospore germination at high temperature may indicate that the upper limit of the physiological thermal tolerance for microstages of *M. pyrifera* is close to 18 °C (Lüning and Neushul, 1978; Amsler and Neushul, 1991; Coelho et al., 2000), which could have severe consequences for southern California kelp populations in the light of the IPCC projections for the next century (IPCC, 2013).

With regard to the effects of pCO₂ on germination of kelp spores, our findings indicate that high pCO₂ (low pH) reduced development of germination tubes. Here, we saw an approximately 13% reduction in the high pCO₂ treatment (pH = ~7.5) compared to the low treatment (pH = ~8.0). Also, examining kelp spore germination under OA context, Roleda et al. (2012) found that low pH (7.61) produced a decrease of 8.7% in the germination rate of kelp spores. The difference in germination rates between studies could be due to different culture methods or differences in adaptation/acclimatization potential between populations. In our study, we used higher temperatures, used lower pH values, and manipulated carbon chemistry by bubbling a CO₂/air gas mix instead of the acid/base manipulation method. Despite the aforementioned differences, the general pattern of decreased germination rates of zoospores at low pH could be explained by the negative effects of increased H⁺ ions on cellular activity, which inhibit cell division and affect cellular metabolism (Sorokin, 1962; Dixon and Kell, 1989; Ragazzola et al., 2012).

Spores from calcifying algae have shown similar responses to the effects of increased pCO₂. For example, in the red alga *Lithophyllum incrustans*, increased CO₂ concentration inhibited the spore production and growth of its early-life-history stages (Cumani et al., 2010). Similarly, in the coralline alga *Phymatolithon lenormandii*, development of early ontogenetic stages was impacted by small pH changes, inducing the increase of abnormalities at low pH (7.5) and a reduction of the growth rate in abnormal thalli compared to the

normal ones (Bradassi et al., 2013). However, there is still a lack of information about the physiological basis of the effects of OA on early microstages of calcifying and non-calcified algae.

4.3. Dormancy

Under unfavorable environmental conditions, the spores of some marine macroalgae have the capacity to persist and survive attached to the substratum in a dormant stage, waiting until their growth is triggered by suitable environmental conditions (Dayton, 1985; Hoffmann and Santelices, 1991; Basso, 2012). Our results indicate that synergistic effects of temperature and pCO₂ may influence the delayed growth (dormancy) in early-life-history stages of *M. pyrifera*. Indeed, ungerminated spores were observed in all experimental treatments but were higher in the combination of low temperature and high pCO₂, which is comparable to the environmental conditions experienced by kelp populations during strong upwelling events (i.e., spring upwelling) in southern California (Yu et al., 2011). In general, dormancy of microscopic early-stages of brown algae has been suggested as a life-history strategy for survival and rapid population recovery after environmental disturbances (Carney and Edwards, 2006; Edwards, 2000; Hoffmann and Santelices, 1991; however see Reed et al., 1997). Unlike the alternate life-history-stages of many organisms that are truly dormant, resting microscopic stages for marine algae likely remain physiologically active and highly sensitive to changes in environmental quality (Hoffmann and Santelices, 1991; Edwards, 2000; Carney and Edwards, 2006). In nature, the majority of the dormant microscopic stages of *M. pyrifera* appear to live at most for weeks to months (Deysher and Dean, 1984; Reed et al., 1997), whereas in laboratory cultures, microscopic stages of kelp have been maintained in a suspended vegetative form for many years (Kain, 1964; Lewis and Neushul, 1994; Carney and Edwards, 2006). Thus, in the context of OA, it may be important to take into consideration the potential role of dormancy as an ecological response of natural populations of *M. pyrifera* to persist in a future scenario of decreased ocean pH.

4.4. Mortality

The viability of spores was the metric that showed the greatest changes in our laboratory experiment. Mortality of spores of *M. pyrifera* was affected by the synergistic effect of temperature and pCO₂. Overall, the number of dead spores increased with elevated temperature at both levels of pCO₂, with the magnitude of the detrimental effects of temperature being much higher in the elevated pCO₂ treatment.

Currently, little is known about the interactive effects of temperature and CO₂ on early-life-history stages of macroalgae. Some attempts using temperature or pH individually have documented drastic consequences

for early developmental stages of marine algae. For example, microstages of *P. lenormandii* and *L. incrustans*, have high susceptibility to high pCO₂, showing increased mortality of germination disks (Cumani et al., 2010; Bradassi et al., 2013). In a similar way, temperature has shown important implications for survivorship of early life stages of kelp species such as *A. esculenta*, with increased mortality of zoospores at temperatures ≥ 16 °C and disk disintegration of young sporophytes at temperatures over 20 °C (Fredersdorf et al., 2009).

Seagrasses and marine macroalgae may be able to partly compensate for the physiological alterations often caused by exposure to elevated temperature (e.g. up-regulation of anti-oxidative machinery and stress response system) or to high pCO₂/low pH (e.g. control ion transport across membranes, increased capacity to store and allocate carbon in different functions), but less capable to maintain their homeostatic and growth function when exposed to both conditions (Hale et al., 2011; Koch et al., 2013). In the case of *M. pyrifera*, the greater mortality that we found under the experimental treatment of high temperature/high pCO₂ could be associated with an inefficiency of cellular repair mechanisms at higher temperatures (Altamirano et al., 2003), that may be exacerbated by high pCO₂. Moreover, it has been suggested that elevated temperatures can have more severe effects on survivorship of spores than in other microscopic life history stages of *M. pyrifera* (Ladah and Zertuche-González, 2007). This may be due to the fact that kelp spores lack a protective outer cell wall, which make them more vulnerable to environmental stressors in comparison to other micro or macroscopic stages (Reed and Lewis, 1994).

4.5. Implication of global climate change on kelp populations

Predictions of the impact of global climate change (GCC) on marine organisms have been often considered in terms of the effects of a single environmental factor on some aspect of organismal performance (Byrne, 2011; Wernberg et al., 2012; Kroeker et al., 2013; Todgham and Stillman, 2013). However, it is now well accepted that GCC is a multi-dimensional problem that involves multiple concurrent factors that can have simple additive (independent) or complex interactive (synergistic or antagonistic) effects at many levels of biological organization and at multiple life-history stages (Hofmann et al., 2010; Boyd, 2011; Byrne, 2011; Bopp et al., 2013; Byrne et al., 2013; Kroeker et al., 2013; Padilla-Gamiño et al., 2013; Todgham and Stillman, 2013). In seaweeds for example, multiple stressor studies have shown that the interaction of UV radiation, ocean warming, large-scale climatic events (e.g., ENSO), increased storm frequencies and salinity variation, impact on fertility, substrate attachment, development, photosynthesis and mortality of its early-life-history stages (Coelho et al., 2000; Izquierdo et al., 2002; Altamirano et al., 2003; Müller et al., 2008, 2009; Fredersdorf et al., 2009; Roleda, 2009). Ultimately, the interaction of these environmental stressors affects the biogeographic distribution, growth and recruitment dynamics of seaweed populations (Reed et al., 1996; Coelho et al., 2000; Buschmann et al., 2004; Graham et al., 2007).

Temporal and spatial variability in the structure and dynamics of climatic and oceanographic processes are driving a major contraction in the distribution of kelp forests worldwide (Deyscher and Dean, 1986; Tegner et al., 1996; Steneck et al., 2003; Buschmann et al., 2004; Halpern and Cottenie, 2007; Hoegh-Guldberg and Bruno, 2010). For example, in natural populations of *M. pyrifera*, high temperatures (>15–17 °C) explain the high mortality of macroscopic stages of the giant kelp during summer (Buschmann et al., 2013), as well as drastic effects on survival of its haploid and diploid microscopic stages (Ladah and Zertuche-González, 2007). However, there are some kelp populations that persist in environments characterized by large fluctuations in ocean pH and temperature (e.g., coastal zone of the California Current Large Marine Ecosystem, CCLME) (Lynn and Simpson, 1987; Ladah and Zertuche-González, 2007). These populations are naturally exposed to values of temperature and pH that exceed, in some cases, those predicted to occur in surface oceans at the end of the century (Yu et al., 2011;

Gruber et al., 2012; IPCC, 2013). This is likely due to the fact that *M. pyrifera* exhibits great phenotypic plasticity in morphological/physiological traits, which allow populations to adapt to specific environments in different regions of the globe (Buschmann et al., 2013). Thus, although future OA and ocean warming may increase mortality of early-life-history stages of kelp populations, it is possible that in environments such as the CCLME, natural selection is acting to favor those individuals that are best adapted to extreme values of these conditions (Coelho et al., 2000).

5. Conclusions

Our results indicate that the environmental change that is expected in coastal oceans may have fitness consequences for a marine non-calcifying foundation species such as the giant kelp *M. pyrifera*. The synergistic effects of high pCO₂ and temperature may affect survivorship and growth during early-life-history stages of this macroalga. The detrimental effects of OA and ocean warming on germination and mortality of *M. pyrifera* zoospores suggest that developmental restrictions (Amsler and Neushul, 1991; Coelho et al., 2000) and the inefficiency of cellular repair mechanisms at higher temperatures (Altamirano et al., 2003) could be exacerbated by low pH. The “dormant strategy” of microscopic stages of the giant kelp could be viewed as a potential ecological response of natural populations of *M. pyrifera* to persist in a future scenario of decreased ocean pH due to more intense upwelling events (Gruber et al., 2012). However, if the intensity and duration of these events increase, the capacity of spores to germinate in the future could be compromised, thereby threatening the health of kelp forest ecosystems.

Nevertheless, because kelp populations in coastal waters of the California Current Large Marine Ecosystem (CCLME) experience strong fluctuations in temperature and pH, with values that are not expected for another ~85 years or longer (Yu et al., 2011), local adaptation and phenotypic plasticity of early ontogenetic stages are likely to play an important role in determining how natural populations of *M. pyrifera* in the CCLME respond to climate change (Kelly and Hofmann, 2013; Pespenti et al., 2013; Sunday et al., 2013).

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jembe.2014.03.018>.

Acknowledgments

This research was funded by an award from the University of California in support of a multi-campus research program, Ocean Acidification: A Training and Research Consortium (<http://oceanacidification.msi.ucsb.edu/>) to GEH. The project was supported in part by the U. S. National Science Foundation's Long-Term Ecological Research (LTER) program (NSF OCE-1232779), specifically the Santa Barbara Coastal LTER (SBC LTER); the authors thank Clint Nelson of the SBC LTER for collection of kelp in the Santa Barbara Channel. In addition, relevant environmental data was acquired with the support of NSF grant OCE-1040960 to GEH and CAB as part of the Ocean Margins Ecosystem Group for Acidification Studies (OMEGAS), a consortium of scientists from different institutions along the U.S. West Coast (see <http://omegas.science.oregonstate.edu/>). During the project, JDGE was supported by a postdoctoral fellowship from Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT-Postdoctoral grant No. 3130381).

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